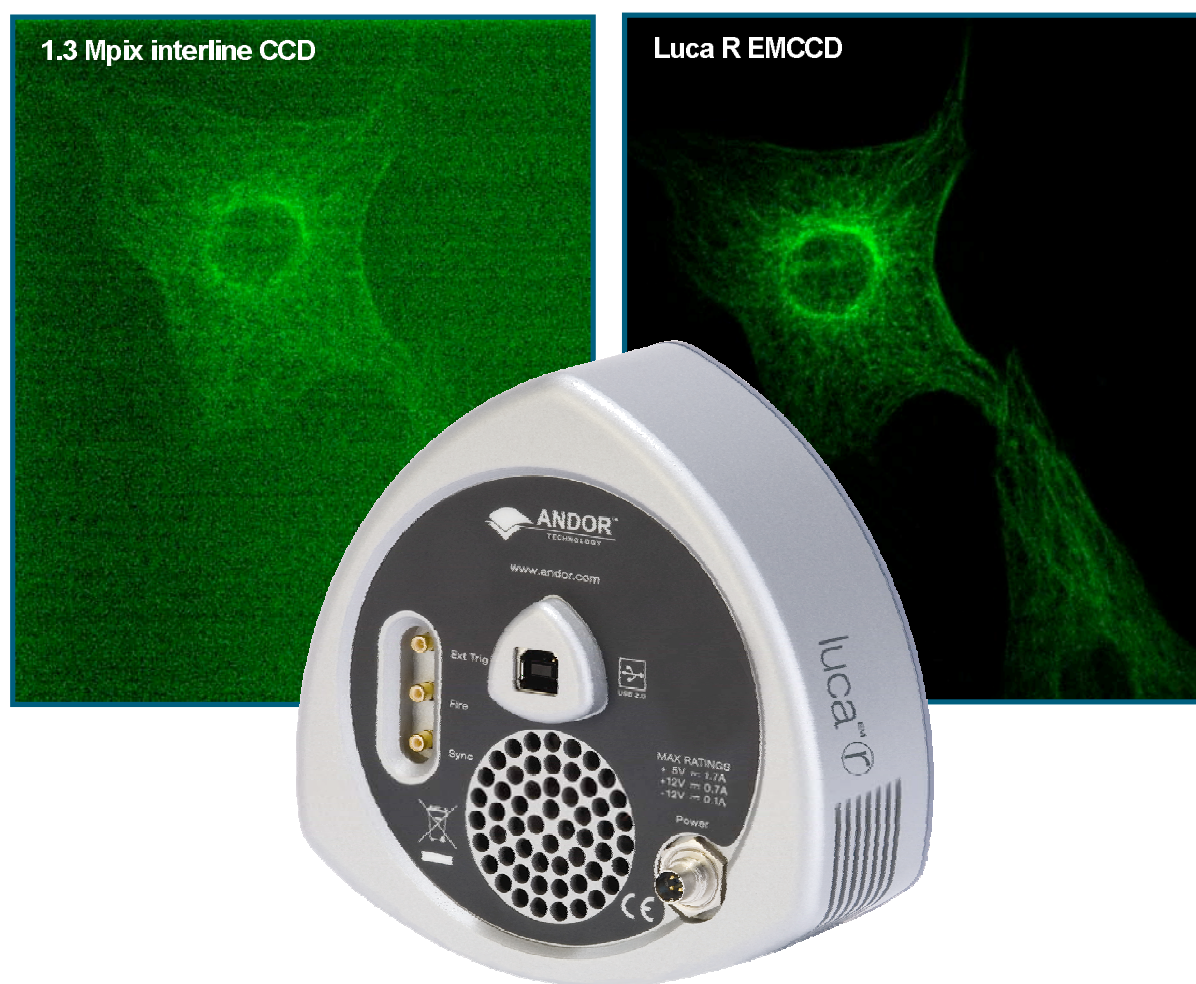

Luca-R EMCCD vs. Interline CCD for cell microscopy applications



How does Andor's Luca-R EMCCD compare to well-established interline CCD for microscopic imaging of cells?

The megapixel sensor format and small pixel size of Andor's new Luca R EMCCD camera presents a novel combination of ultra-sensitivity, resolution, field of view, and above all, flexibility. The EM gain feature of this camera offers the unique advantage to use the Luca R in light-starved conditions, in which current leading interline CCD sensors may not deliver optimal image quality.

In this case study we compared the sensitivity and resolution performance of the Luca-R against the highest specification interline CCD on the market, in a cell microscopy application:

1. **Luca R EMCCD** – 1 Megapixel EMCCD frame transfer sensor; $8 \times 8 \mu\text{m}$ pixel size; $< 1 \text{ e}^-$ read noise (with EM gain); 12.5 frames/sec (max.); -20°C cooling; USB 2.0 interface to PC.
2. **Interline CCD camera** – Very common 1.3 megapixel interline CCD sensor; $6.45 \times 6.45 \mu\text{m}$ pixel size; optimized to deliver 5.5 e^- read noise; 11 frames/sec (max.); -30°C cooling; Firewire interface to PC.

Each camera comparably offers $> 60\%$ QE at the test emission wavelength:

We demonstrate that:

- Under low light imaging conditions, the Luca-R EMCCD convincingly outperforms the interline CCD, delivering much better signal contrast and, as a result, better resolution.
- Under bright signal conditions, the interline CCD offers somewhat better image quality – this is due to the now dominant influence of smaller pixel size.
- Even under conditions of 'moderate' intensity, better spatial resolution can be achieved from higher sensitivity EMCCD, despite larger pixels. This is afforded through enhanced contrast between signal and background noise, thus better definition of fine structural features.

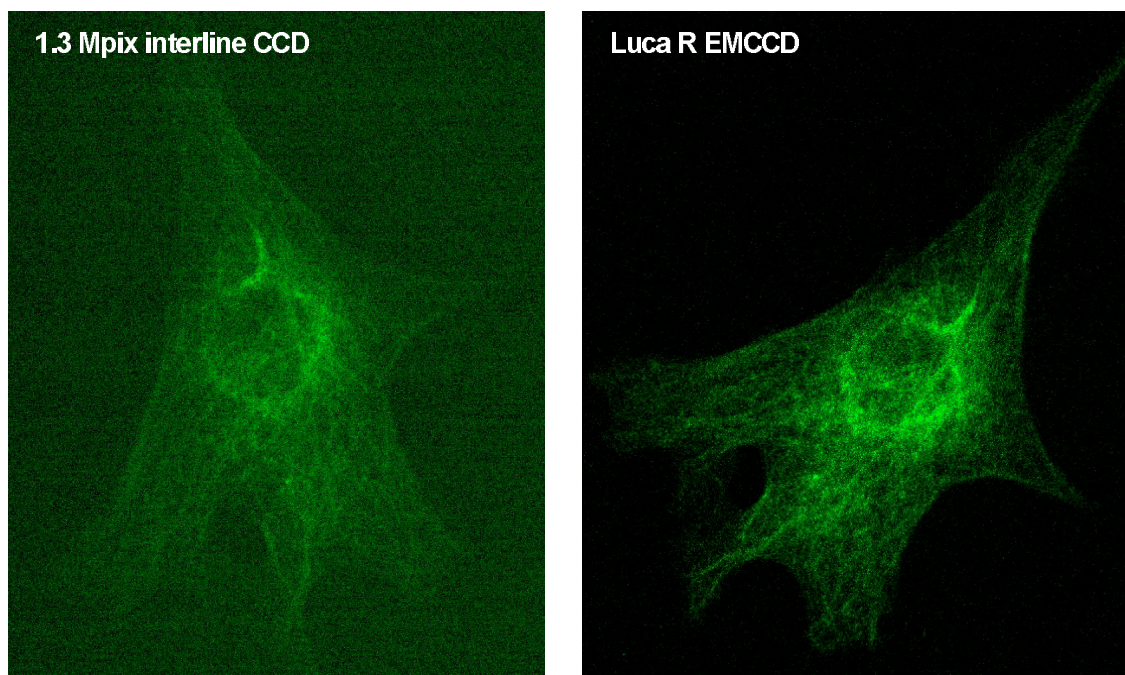


Figure 1 – 1.3 MPix interline CCD vs. Luca-R EMCCD under low light imaging conditions. 100 ms exposure time was used and 10% of available laser power (approx. 2 mW at 488nm) illuminated the sample. Read noise of the interline camera is $5.5 \text{ e}^- \text{ rms}$ (i.e. up to $\sim 20 \text{ e}^-$ peak-to-peak) and is dominant in that image. Note that the images are rotated slightly relative to each other.

IMPLICATION FOR LIVE CELL IMAGING

Since microscopy on **living cells** will at some stage ultimately push the user to low-light conditions, then the **flexibility** of the Luca R to operate **well under bright conditions**, and **extremely well under low light conditions** makes it the better choice for live cell work in general.

Test conditions

Luca R and CCD X were compared on an inverted microscope using confocal illumination through a spinning disk unit (CSU-22, Yokogawa) to test sensitivity performance and resolution under different signal strength conditions. 488nm laser excitation was employed and the fluorescence of a green emitting fluorophore was detected from a standard Bovine fixed immunofluorescent cell sample (Invitrogen). A x60 objective lens (oil. NA 1.42) was used with additional x1.2 magnification between microscope and the camera.

These two cameras were compared across a range of illumination intensities, repeated over a number of cells. All tests were designed to weight both cameras' performance in terms of signal-to-noise, spatial resolution and their impact on fluorescence microscopy applications.

In the series of tests, both cameras were subject to measurements in the following light conditions:

1. **Low-light conditions (short exposure and/or low laser power), typical of demands imposed by live cell measurement.**
2. **Light-rich conditions (longer exposures and/or higher laser power).**
3. **Intermediate signal, i.e falling between conditions 1. and 2.**

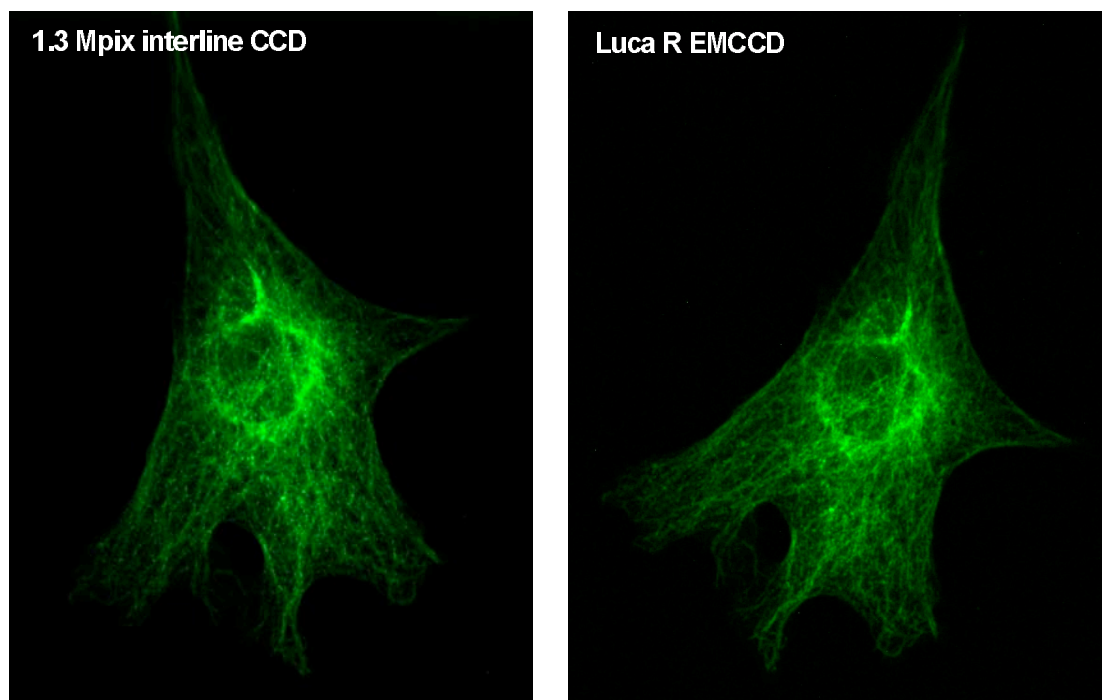


Figure 2 – 1.3 MPix interline camera vs. Luca-R under higher signal conditions. Signal intensity during this test was 40x higher than used in Figure 1. The 5.5 e⁻ rms of the interline CCD is much less of a factor, and the smaller pixel size now afford a somewhat higher resolution image compared to the EMCCD Luca R.

Results

Under restricted signal conditions, derived through use of low laser power and/or short exposures, the Luca R significantly out-performed the interline CCD sensor, both in terms of Signal-to-Noise *and* resolution (because the latter is highly influenced by the former) as shown in **Figure 1**. Application of EM Gain of the Luca-R serves to amplify the weak signal above the read noise floor, thus drastically improving contrast and definition of fine structural features inside the imaged cell.

The lower sensitivity of the interline CCD camera is due primarily to the influence of the read noise floor (5.5 e^- rms under the readout speed/pre-amplifier gain combination used). Its sensitivity (and therefore resolution) has also been affected by the relative pixel size, the Luca-R pixel having greater area for light collection compared to the interline CCD pixel.

When brighter conditions were employed, such that the obtained signal was stronger and well clear of the read noise floor, the interline sensor with its smaller pixels delivered sharper resolved microscopic images as shown in **Figure 2**. Such intensities are typical for signals often found in bright widefield fluorescence or DIC microscopy. Whenever one goes further towards lower light intensities, the noise on the interline sensor will contribute more and more to the image, and the resolution advantage of pixel size is eventually lost.

This scenario can still be seen in an 'intermediate' signal, whereby the light levels are neither very low nor very high, as offered in **Figure 3**. In this case the Luca-R can still be argued to exhibit superior resolution performance. In such a case, the interline camera that should offer superior spatial resolution by virtue of Nyquist oversampling of the diffraction limit, can still suffer from a readout noise contribution which hampers the ability to resolve fine intracellular detail.

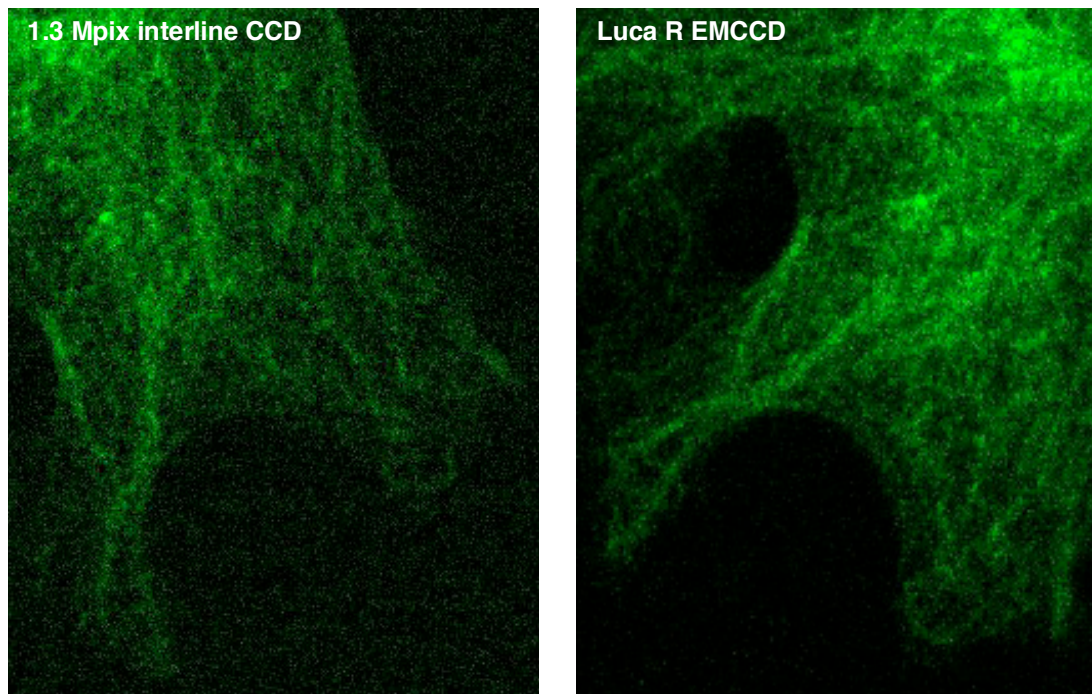


Figure 3 – Zoomed-in fragments acquired with the interline camera and Luca-R in images recorded under 'intermediate' signal conditions. Signal intensity was 4x higher than used in Figure 1. The 5.5 e^- rms of the interline sensor still impinges on image quality, resulting in loss of definition.

The ability of the Luca-R to perform markedly better in low light conditions is in line with the theoretical calculations of Signal-to-Noise ratio for the two different sensors used in these cameras, as illustrated by the plot shown in **Figure 4**. At photon fluxes lower than ~ 300 photons per $8\ \mu\text{m}$ pixel (corresponding to ~ 195 photons per $6.45\ \mu\text{m}$ pixel), the read noise of the interline sensor starts to contribute to and 'pull down' the S/N curve for that sensor, such that at low photon flux values there is a marked difference in S/N between interline cameras and the Luca-R with EM gain. Note that for other camera manifestations of this interline CCD sensor exhibiting even higher read noise, this break point would appear further up the curve still.

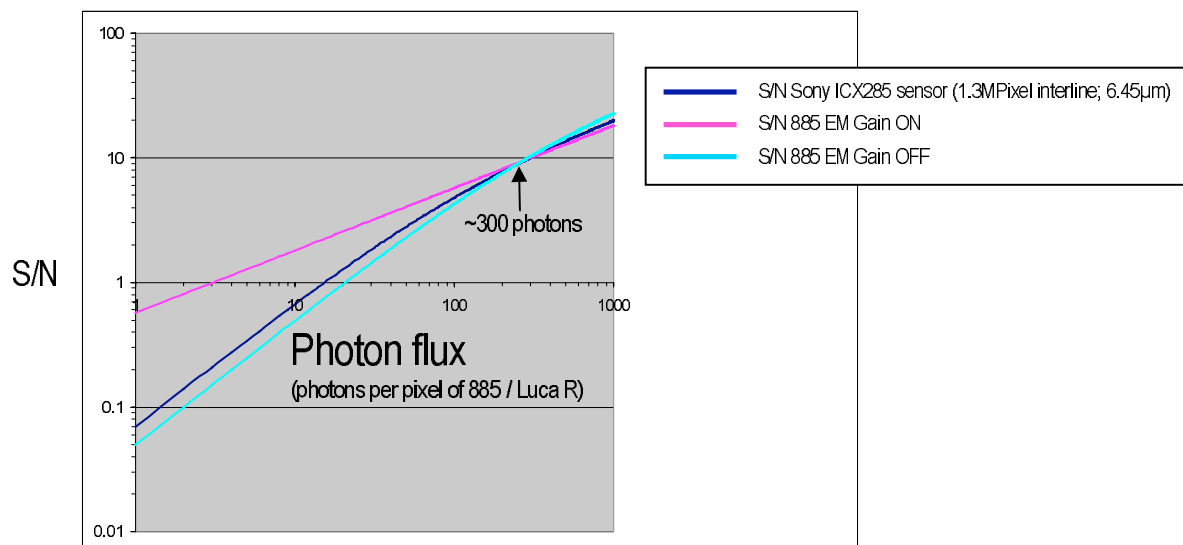


Figure 4 – Plot of Signal-to-Noise vs. Photon Flux for the Luca-R vs. a camera containing the 1.3 MPix interline CCD sensor, Assuming 5.5 electrons rms read noise for the latter and accounting for both pixel size and QE differences at 550 nm. The Luca-R shows significantly higher S/N in the low light regime with EM gain applied.

Please note also that only *moderate levels* of EM gain are needed for the Luca-R to approach the optimal S/N curve (shown in pink), in fact between x100 to x200 EM gain would suffice. Applying moderate EM gain has the advantage of improving the dynamic range of the measurement.

Conclusions

- It is clear that in all experimental conditions involving low light levels, Luca R delivers superior images and cannot be out-performed by a camera incorporating the interline CCD sensor optimized for lowest possible noise, as in this case.
- Luca R offers flexibility that the interline CCD chip camera cannot attain because of lack of the EM gain. Often in live cell imaging the users will ultimately push to low light conditions (or higher frame rate) at some stage or other. Luca R will operate well under bright conditions, and *extremely* well under low light conditions.
- All this makes Luca-R the much better choice for live cell work whereby it's raw sensitivity and flexibility positions it ahead of the interline CCD sensor.

How do I choose between Luca-R and iXon+ 885 EMCCDs?

Each of these camera types contain the same megapixel EMCCD sensor and therefore each will out-perform the 6.45 μm pixel size interline CCD under low light conditions.

Further refining your decision to choose between these two EMCCDs can therefore be dependent on the following factors:

- **Speed** - iXon+ 885 delivers frame rates up to 30 full frames/sec (as opposed to 12.5 frames/sec of Luca-R), and much faster under sub-array/binning.
- **Cooling** - The deeper vacuum cooling of the iXon+ 885 (down to -95°C) minimizes the dark noise under EM-amplification, which can benefit imaging modalities with very low photon background.
- **Size** – the highly compact Luca-R (also available in an extremely compact square housing) may suit better to experimental constraints.
- **Interface** – similarly, the USB 2.0 interface of the Luca-R may be better suited to some measurement environments, such as when restricted to laptop use.
- **UV responsivity** – The Luca R does not perform well below 400nm, whereas the iXon+ 885 offers admirable QE performance in the UV range.
- **Budget** – The Luca-R is the lower price of the two. Can particularly make a difference when the application requires a synchronized multiple camera solution!